Chapter 17

INTEGRATED AND EXPERIMENTAL APPROACHES TO THE STUDY OF GROWTH OF ORGANISMS AROUND ROOTS

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Abstract

Emphasis is placed on the need to devise experiments which answer critical questions on the growth of micro-organisms on roots in soil. A conceptual framework identifies the main components of the system and leads to simple definitive experiments on these components. Some of these experimental methods are indicated. The power of quantitative models of growth in the rhizosphere is discussed.

It is suggested that organisms which colonize roots later become major components of the soil microflora on organic matter which acts as a prime source of inoculum for roots in the following season and may lead to suppression of pathogens. Changes in soil microflora and build up of antagonists to pathogens are probably quickest under crops and conditions leading to high root concentrations. Root concentration is discussed in relation to fungus propagule size and growth to the root.

Introduction

One of the challenging questions facing the soil microbiologist and plant pathologist is whether we can control the microbiology of the rhizosphere. The plant pathologist seeks to depress the colonization of roots by pathogens and encourage antagonists to them. Soil microbiologists often wish to introduce highly selected symbionts such as mycorrhizal fungi or rhizobia into a soil containing organisms already well adapted to the particular soil; shortfalls in plant response to inoculation by 'biological control' of symbionts can be just as important as traditional plant diseases (Bowen, 1978).

We are a long way from being able to manipulate the whole of the rhizosphere population; the only realistic approach at present is to examine experimentally the ecology of particular organisms in order to manage that organism.

The biology of the rhizosphere was reviewed by Rovira and Davey (1974). Microbial colonization of roots was reviewed recently by Bowen and Rovira (1976) and in this paper I suggest methods and approaches to gain a better understanding of the kinetics and interactions of organisms growing around roots and seeds in their *real* environment, the soil.

The rhizosphere environment

Chemical

Several sources of organic nutrients from roots have now been defined by Rovira *et al.* (1979); these encompass a "blur" of exudates leaking from cells, active secretions of low molecular weight compounds and of plant mucilages, lysates from autolysing epidermal cells and sloughed root cap and epidermal cells. Prior to this decade most studies on substrates for microbial growth from roots were from solution-grown plants. We now know that amounts measured were not only extremely low (0.1-0.4% of the photosynthate) but also the composition of the exudate was different from that of soil-grown plants. For example, Papavizas and Kovacs (1972) found fatty acids needed for germination of *Thielaviopsis basicola* propagules occurred in exudates from plants in sand culture but not from plants in solution culture, and Barber and Martin (1976) found that roots of wheat grown to the early tillering stage in soil lose 5-10% of the photosynthetically fixed carbon under sterile conditions and 12-18% under non-sterile conditions. Martin (1977) reported up to 39% of carbon translocated to wheat roots was released and although much of this was respiratory carbon dioxide, he considered that a large component was due also to lysis of root cells. Rovira (1956) showed that root exudates stimulate the growth of bacteria, but there is an urgent need to experimentally relate microbial abundance to the amounts of materials lost by the root.

Stotzky and Schenck (1976) drew attention to the importance of biologically active organic volatiles released by seeds and the subterranean parts of plants. These include various alcohols, aromatics, terpenes, ethylene, aldehydes and acetone. Volatile organics have been shown to inhibit and to stimulate germination of various fungal spores. Many such studies are performed in laboratory conditions and their importance in soils needs evaluation. Their hypothesized importance rests on their possible biological regulatory activity, their more rapid diffusion through soils than that of solutes and that they can act as the sole carbon sources for some bacteria.

Apart from a wide range of sugars, amino-acids, organic acids and other compounds which can be used by many microorganisms, roots and seeds release many unusual compounds which may be used by only a few organisms or may be toxic to many others (Rovira, 1965). Such compounds could play a large part in determining the composition of the microflora around roots and germinating seeds. Krupa and Fries (1971) have suggested that volatile terpenes produced by *Pinus sylvestris* roots, (and increased several-fold by infection with the mycorrhizal fungus *Boletus variegatus*) reduce competition to the mycorrhizal fungus and may reduce growth of pathogens such as *Fomes annosus*. This hypothesis is attractive but needs experimental verification. Great variation occurs in the types of compounds such as phenols and terpenes produced by roots and seeds and in their effects on various micro-organisms, e.g. Wahab (1977) found differences with soybean variety - *Rhizobium* strain combinations in *Rhizobium* sensitivity to seed coat toxins. Establishing particular beneficial organisms in the rhizosphere may be possible by breeding plants producing a high percentage of unusual compounds and also introducing the ability to use these compounds into the selected beneficial micro-organism. Cronquist (1977) suggested the production by plants of new secondary metabolites acting as insect repellents has been important in the evolution of major new groups of dicotyledons. Similar arguments could apply to pathogens and to selection of rhizosphere organisms compatible with the plant and perhaps we could develop this approach further.

The pH and the inorganic composition of soil solution at the root surface can be quite different from that in soil due to the charges on the surface and differences between ions in movement to the root, absorption by the root, accumulation at the root and loss of ions from the root. This can be further modified by fertilizer practice, e.g. Smiley (1974) found differences of up to 2.2 pH units in the pH of the rhizosphere of wheat for NH_4^+ vs. NO_3^- fed plants. The pH in the rhizosphere was up to 1.2 units different from that of non-rhizosphere soil. He pointed out (1975) that in the critical pH range of a fungus, much smaller pH differences may profoundly affect its growth and also the composition of the rhizosphere microflora.

Physical

Scanning and transmission electron microscopy (e.g. Foster and Rovira, 1976; Rovira and Campbell, 1974, 1975) have given an understanding of the physical nature of the root-soil interface. Micro-organisms form colonies in the mucigel, a gelatinous material including plant mucilages, bacterial cells and their metabolic products, colloidal material and organic matter from soil. It is thought the mucigel is important in maintaining a pathway for nutrients and water between root and soil during diurnal root shrinking and swelling (Rovira *et al.*, 1979). Microbial entry into older, collapsed cells often occurs (Foster and Rovira, 1976).

It is paradoxical that most studies of growth kinetics of micro-organisms have been in stirred solutions but growth in nature usually occurs at surfaces, where growth rate, yield, and saturation constants are quite different from those in solution (Hattori, 1976). Microbial growth in films in other systems (Bazin *et al.*, 1976) such as in trickling filters has many factors in common with that in the rhizosplane, e.g. growth in micro-colonies in mucilage, attachment by production of microfibrils, and possible control of growth by oxygen diffusion through the microbial film.

A conceptual framework for rhizosphere biology

Rhizosphere micro-biology has developed in a somewhat fragmented way ; for example, of the many studies of root exudates which have been performed few relate root exudates experimentally to microbial growth. Part of the difficulty is the large task in encompassing both plant physiological and micro-biological studies simultaneously, but I suspect part of the fragmented approach to rhizosphere biology comes from lack of enunciation of a conceptual or theoretical framework. A conceptual framework, even a qualitative one, formalizes the way we think the system works and indicates the various areas which may be manipulated to

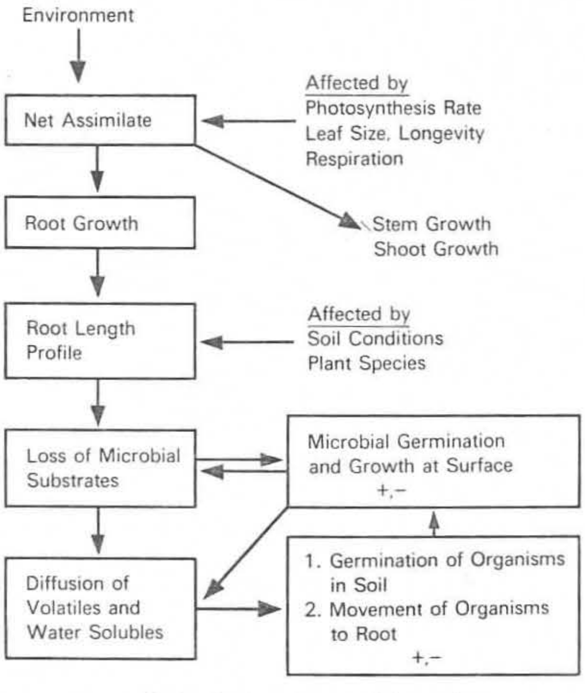

Fig. 1 A conceptual model of the plant-rhizosphere system

affect microbial growth and to increase biological control of pathogens. Thus even the simple framework of Figure 1 formally indicates:

1) Control of microbial growth could be obtained in three processes, viz: germination of the propagule, movement to the root and growth at the root surface. Research workers should not prejudge the alternatives by concentrating on only one of these processes, e.g. rhizosphere growth, without very good reason.
2) The possibility that inhibitory substances as well as stimulatory ones may come from the root.
3) That substrates (and surfaces) for microbial growth are influenced (qualitatively and quantitatively) by the root and thus factors affecting net assimilation, and distribution of assimilates (see Bowen and Cartwright, 1977) may have large effects on microbial activity. This suggests several avenues for genetic and physiological manipulation of exudates and highlights the need for whole plant approaches to understanding disease expression and symbiotic response (Bowen, 1978). Some physiological manipulation of the rhizosphere has been shown in experiments by van Vuurde and Schippers (1975), and other workers, by foliar applications of urea.

Experiments with soil-grown plants

The artificial conditions of many experiments, e.g. exudate studies in solution, and spore germination, microbial growth and interactions on agar, have little relation to the behaviour of micro-organisms in the rhizosphere (e.g. Broadbent *et al.*, 1971; Bowen and Theodorou 1973, 1979). If we are to really understand the kinetics of microbial growth around roots we have no alternative to studying plants in soil - that is the real world, where we must make our measurements and conduct our experiments. A number of relatively simple but valuable experimental approaches are already available and more will certainly be developed to answer specific questions once they are enunciated.

Although movement of organisms through soil to roots and their growth there is important to understanding the epidemiology of root diseases, it is surprising just how little experimental data exist on such factors. Pathologists and soil microbiologists have been slow to adapt concepts and methods of assessments of populations and their distribution and migration, which have guided quantitative ecology and population biology in other fields. Yet there are very real parallels and analogies with growth of organisms on roots and we might profit by examining concepts of primary colonizers, of weed species, and of succession and stability of ecosystems which have been studied in other fields.

We must examine the ecology of particular pathogens or of beneficial organisms, experimentally varying important factors to understand their effects. The various components of the system must first be broken down into smaller discrete ones (e.g. Figure 1). Questions can then be asked which are capable of simple effective experimentation. Many methods of observation exist, including the use of selective plating media and using direct microscopy to trace particular organisms by means of distinguishing morphological characters, fluorescence microscopy, fluorescent antibody methods and radioactive isotopes (Bowen and Rovira, 1976; Mayfield, 1977). Some of the approaches which have been used to examine areas of Figure 1 are indicated below (see also Bowen and Rovira, 1976). An extension of such studies with organisms of particular interest will build up a picture useful in understanding their epidemiology and their possible management.

Propagule germination

Accurate placement of propagules in soil at set distances from roots to study the influence of roots on germination and growth of propagules is not difficult. Usually the root can be restricted to a known position by use of mesh small enough to restrict roots and root-hairs but allowing penetration by the micro-organisms (Wildermuth, 1977). Recovery of smaller propagules from soil may be difficult but can be assisted by sieving-flotation techniques coupled with identification by fluorescence antibody methods (if necessary) (Malajczuk *et al.*, 1978). Recovery and observation of spores can be facilitated by attaching them to a glass slide or polyethylene tape but this can introduce artefacts due to surface effects. Conditions for germination of vesicular-arbuscular mycorrhizal spores (in this case greater than 200 μm) have been studied by placing them in soil in 1 cm^3 cages of stainless steel mesh of smaller aperture than the spores, burying the cages, then later recovering spores by wet sieving the soil from the cages (G.D. Bowen, unpublished).

Growth through soil

Placing propagules of pathogens at known distances from roots and examining the roots for infection (Henis and Ben-Yephet, 1970) can considerably help our understanding of epidemiology and the organisms likely to colonize a root. There are many simple but important studies yet to be made on the relation of propagule size to the distance the propagule can grow to a root and the effects of factors, such as other soil organisms, soil moisture, compaction, pH and temperature, on growth through the soil. Using stainless steel mesh of 40 μm aperture to prevent root penetration into soil in which propagules of *Gauemannomyces graminis* var. *tritici* (G.g.t.) were placed at known distances from the root, Wildermuth (1977) has shown that a major component of suppression of take-all disease of wheat occurs by decreasing the growth of the fungus through soil to the root, rather than control at the root surface.

Growth on the root

Factors to be considered in examining growth of micro-organisms in the rhizosphere include:

1) the effects of various plant, soil and environmental parameters on growth and spread of the micro-organism compared with that of other organisms available to occupy the root,
2) the interactions between organisms on the root surface,
3) whether an organism "arriving" late at a root, (having grown through soil) can impose itself on an existing population.

Although the final test is in natural field soil, the relative importance of particular factors such as pH, soil temperature and moisture may often be assessed by studies in sterile soil. Experimental comparison between sterilized and non-sterilized soils at critical points can then indicate the importance of that variable on the test organism via its effects on the rest of the soil microflora or microfauna.

Growth rates on roots

Two complementary approaches to examining the development of organisms on roots are (1) to study different segments of known age on the same root and (2) to examine growth on a nominated segment of root, by using a time series of harvests.

If one is interested only in the capacity of substrates from roots to support microbial growth, it is more direct to measure microbial growth than to collect and analyse "exudates" for sugars, organic acids, amino-acids etc. Furthermore, growth of organisms at a particular segment of root is likely to be a far more practical and sensitive way to estimate relative amounts of substrate coming from that segment. This technique was used by Bowen and Rovira (1973) who grew *Pinus radiata* roots in sterile soil for 10 days, washed them free of possible adhering microbial substrate and planted them to non-sterile soil. Daily counts from the original apical centimetre, the fifth and the tenth centimetre from the apex all showed growth curves of the type shown in Figure 2. This did not identify the sources of the substrates; in the basal area they may have been from senescing and sloughed epidermal cells rather than from secretions from intact cells which would have occurred in the apical centimetre. An initial rapid growth phase occurred ("a" in Figure 2) the slope of which can be regarded as an "intensity" factor indicating the richness

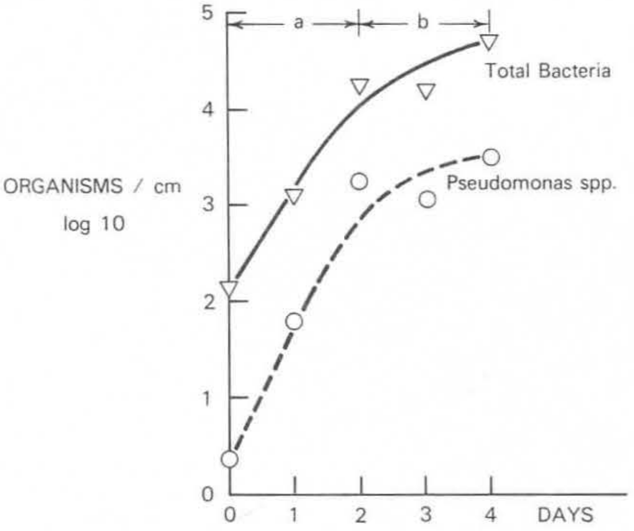

Fig. 2 Growth curves of total organisms and of pseudomonads on roots of *Pinus radiata* 0.5-1.5 cm from the apex. Sterile roots were planted into natural soil. a = intensity factor, b = capacity factor (see text).

of the substrate for microbial growth and then a slower phase ("b") the level of which can be regarded as a "capacity" factor in which substrate supply balanced maintenance requirements of the bacteria. The slow increase in cell number in the second phase is probably due mainly to migration of cells to uncolonized parts of the segment and multiplication there.

Bowen and Rovira (1976) reported "generation times" (or "doubling" times, Barber and Lynch, 1977) on the root of 5.2 h for pseudomonads (77 h in soil) and 39 h for *Bacillus* species (greater than 100 h in soil). This way of recording growth in the rhizoplane and soil is consistent with the terminology of other areas of population dynamics, and it is a more correct way to compare growth in different systems, e.g. at a surface (the rhizoplane) and in a volume (the soil).

Wide differences occur in growth rates of different strains of the one species of micro-organism in the rhizosphere, e.g. a major part of interstrain competition between rhizobia in nodule formation occurs during establishment in the rhizosphere. Wildermuth (1977) found the length of runner hyphae of G.g.t. on wheat roots was 37.3 mm from the point of inoculation after 20 days but that of *Gaeumannomyces graminis* var. *graminis* (G.g.g.) was only 3.3 mm; thus a high population of G.g.g. would be needed to compete with G.g.t. for space on the root and the question is how to achieve this. Table 1 shows that two strains of *Pseudomonas fluorescens* inoculated to roots of *Eucalyptus seiberi* grew at quite different rates, and a third did not increase its population. The growth of strain El_3 on *E. seiberi* was 3 times that on *E. globulus*, but strain J_7 grew to the same extent on both species. At present we do not know the reasons for these differences in growth between strains.

TABLE 1

Growth of three strains of Pseudomonas fluorescens *on roots of* Eucalyptus seiberi

Strain	At inoculation	Bacterial Population ($\log_{10}\text{cm}^{-1}$ ° after 4 days 20°C
J_7	4.01 (0.23)*	5.80 (0.23)
C_5	4.36 (0.09)	4.62 (0.26)
El_3	4.62 (0.16)	7.20 (0.04)

* Mean (± S.E.) of 5 replications of the segment 1-2 cm from the apex. Roots were planted in non-sterile soil at 25°C.

Migration of bacteria along roots has been experimentally examined in *Pinus radiata* by inoculating roots 2.5 cm behind the root apex using a 1 mm^2 filter paper impregnated with *Ps. fluorescens*, replanting the roots to soil, and counting the organisms in successive centimetres from the point of inoculation at various times. At drier than 0.2 bar suction, growth and migration were severely restricted. Such studies show the potential for migration under various conditions, but it may be very much less in non-sterile soils, if new populations from soil develop quickly enough to occupy most routes of migration.

Growth rates of fungi along roots are usually measured from a point of inoculation and the effects of a number of soil factors on ectotrophic growth of mycorrhizal fungi have been reported by Bowen and Theodorou (1973). A line intercept method (derived from methods for measuring root lengths) was used by Rovira *et al.* (1974) to measure the amount of fungal growth along roots and Wildermuth and Rovira (1977) verified the accuracy of this with G.g.t. hyphae on wheat roots. Another method for measuring microbial abundance on roots, not yet used but capable of adaption, is that of image analysis.

Under good conditions for root growth, the roots can usually outgrow fungal hyphae (which may catch up to the tip when root growth slows, e.g. with ectomycorrhizae) (Bowen and Rovira, 1976). This allows other organisms to colonize the new root along the cell junctions, the preferred routes of migration, thus slowing down hyphal growth further (Bowen and Theodorou, 1973).

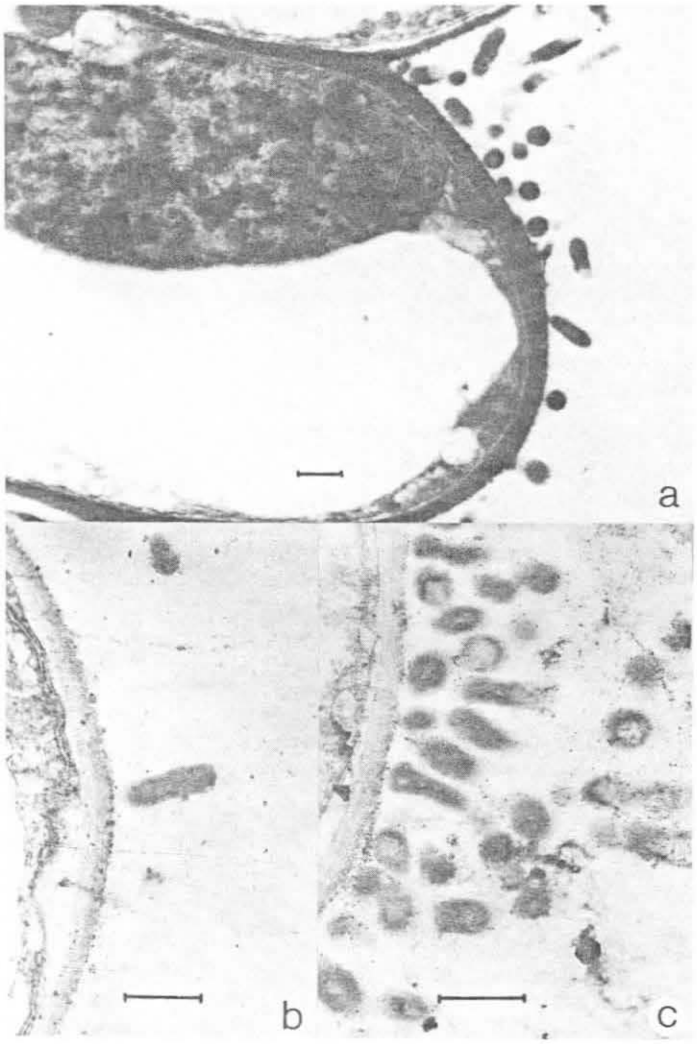

Fig. 3 E.M. study of *Pinus radiata* rhizosphere in soil a) bacterial growth in mucigel in cell junctions, b) surface colonization of a young cell, host cytoplasm intact c) colonization of a senescent young cell nearby (Bowen and Foster, 1978). Bar = 1 µm.

Microbial distribution on roots

Application of standard ecological techniques has shown the distribution of micro-organisms on roots to be non-random (Newman and J. Bowen, 1974) and to be preferentially in the junctions between cells and on individual senescent cells in even quite young parts of the root

(Bowen and Rovira, 1976; Bowen and Foster, 1978) (see Figure 3). Whether the latter senesce and are then heavily colonized or whether subclinical pathogens first cause the cells to senesce, is not known but the answer could be found by an experimental approach. The cell junctions (probably the sites of greatest exudation) are the main routes of migration (Bowen and Foster, 1978) and, although colonization of the cell junctions is not continuous, their occupation can seriously retard later ectotrophic growth of fungi.

The colonization of cell junctions as the most conducive places for growth, first observed by Rovira (1956), was experimentally verified by uniformly inoculating roots with a thin layer of *Ps. fluorescens* and then examining growth after 2 days incubation in sterile "Perlite" (see Figure 4), (G.D. Bowen, unpublished). This inoculation was performed by dipping roots briefly in 1% water agar at 35°C, containing a suspension of the test organism which, in gelling, prevents migration of the bacteria. The method is amenable to use in plant pathology studies such as examining spore germination at various parts of a root. Frenzel (1960) used a similar method with amino-acid requiring mutants of *Neurospora crassa* to detect exudation of particular amino-acids.

It takes some three days under highly favourable conditions for microbial cover of new root growth to become

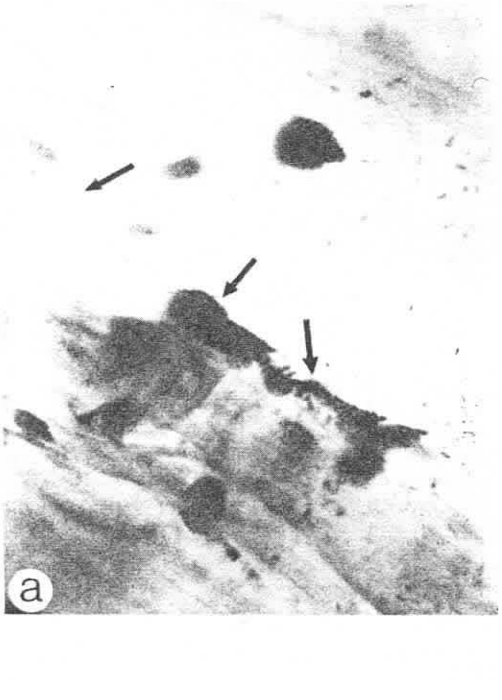

Fig. 4a

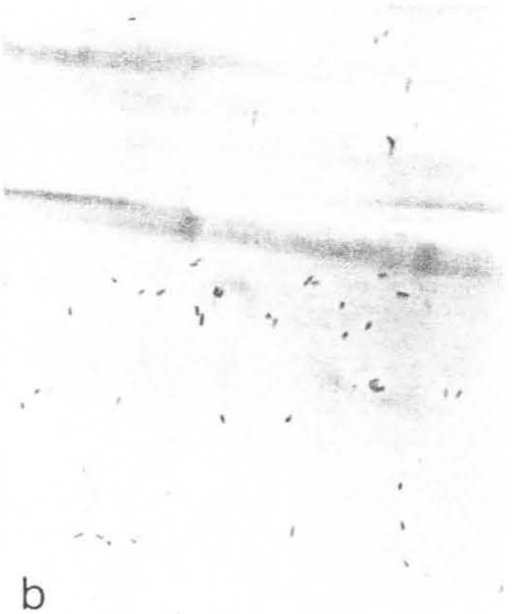

Fig. 4 Experimental study of preferred sites of microbial growth. Sterile roots of *P. radiata* (a) or bundles of sterile glass fibres (b) were uniformly inoculated with *Ps. fluorescens* in 1% water agar. After 2 days no growth occurred on the fibres and growth was mainly at cell junctions (arrowed) of the root (G.D. Bowen, unpublished).

more than a few percent, and even considerably later the cover of the root is usually only 8-10% (Bowen and Rovira, 1976). It is difficult therefore to envisage that antagonists in the rhizosphere will be important in preventing infection by rapidly invading organisms such as *Phytophthora cinnamomi*.

The incomplete microbial cover of roots also allows space for late arrivals or slow growers to colonize, although many sites have already been occupied by other organisms. Several sources of substrate may be available for late colonizers (Bowen and Rovira, 1973) but one of the most effective ways for a microbial species to obtain a "foothold" would be for it to increase permeability of the root in its vicinity. There is considerable scope for this by production of hormones or of mild toxins.

Organisms associated with soil organic matter are a prime source of inoculum for roots, suggesting a tightly coupled relationship between organisms colonizing the roots of a crop and the primary colonizers of the next season's roots (Bowen and Rovira, 1976). This leads to establishment of a new microbial equilibrium within a few years of changing a cropping system. It follows that organisms heavily colonizing this year's roots will be a major source of the suppressors of spore germination, zoospore production and fungal growth through soil in the next crop. Deacon (1973) suggested grass leys could be used to increase populations of *Phialophora radicicola* var. *graminicola* (P.r.g.), antagonistic to G.g.t. and Slope *et al.* (1978) showed P.r.g. was abundant on wheat roots only after grass. Crop rotations aimed at reduction of disease could be oriented to increasing antagonists rather than to the longer rotations which are often needed if one is aiming to decrease propagule numbers in soil.

The results in Table 2 indicate some organisms may have little effect on a fungus (*Bacillus* sp. 1), another may depress growth (*Ps.fluorescens*), and another may stimulate it (*Bacillus* sp. 2). Some fungi (e.g. *Thelephora terrestris*) were relatively insensitive to the other organisms and this may have ecological significance as *Th. terrestris* is one of the most common mycorrhizal fungi in pine forests of Australia and U.S.A.

TABLE 2

Rhizoplane interactions between mycorrhizal fungi and bacteria (Bowen and Theodorou, 1979)

	Length root colonized by fungi (mm) Bacteria present				
	Exp. 1			Exp. 2	
	0	*Ps. fluorescens*	*Bacillus* sp. 1	0	*Bacillus* sp.2
Rhizopogon luteolus	27.6	8.3	23.7	20.7	34.1
Suillus luteus	16.2	3.2	8.7	19.1	26.2
Thelephora terrestris	22.4	12.9	18.1	12.1	11.2
Corticum bicolor	17.2	3.2	15.3	15.8	27.1
Pisolithus tinctorius	6.9	5.7	4.2	6.9	1.9
L.S.D. P <0.05	5.4			6.2	
<0.01	7.1			8.2	

Experiments were conducted in sterile soil for 4 weeks at 20°C day. (12 h), 16°C night.

The suppression by *Ps. fluorescense* was *probably* due to antibiosis as other pseudomonads multiplying to the same extent did not give as strong a depression. However, depressions of growth may also be due to competition for space or nutrients, and desirable traits for a suite of organisms to reduce ectotrophic growth of a pathogen would be for them to use a wide variety of substrates, grow rapidly, and be compatible with each other.

Wildermuth (1977) showed G.g.t.-suppressive soil did not prevent the growth of G.g.t. hyphae on the root. On the other hand, Rovira and Campbell (1975) demonstrated lysis of G.g.t. hyphae to occur on the roots of wheat growing in sand inoculated with *Pseudomonas* spp. - possibly such lysis and prolific bacterial growth on the hyphae was with older hyphae. Lysis and decomposition of fungal cell walls will enrich the roots with organisms capable of giving specific suppression of disease in subsequent seasons.

Quantitative models

Reliable quantitative models are a considerable advance on the conceptual model of Figure 1 in that 1) they quantify changes on a system with time and with variation of conditions, 2) they evaluate the relative importance of factors controlling the system and this indicates the most important factors to attempt to manage genetically or agronomically, and 3) the enunciation of a model indicates the type and form of data which experimenters should be obtaining - much time and energy can be wasted in agricultural and microbiological research by collection of inadequate, inconsequential or irrelevant data.

One "approach" to model building is to be overwhelmed by the many gaps in information and to embark on enormous data collecting expeditions. I suggest this is defeating one of the purposes of models. A more effective strategy is to build a relatively simple model but one detailed enough to include the major components considered to be important. Often the data will be poor or non-existent and the modeller must assume values and relationships from "experience". This is not wrong as long as he/she remembers it is an assumption. When a reasonably convincing output is obtained, a sensitivity test should be conducted by varying each component systematically. A small change in output from varying a particular component suggests there is little scope in trying to manage that component. A sensitive component though indicates the assumptions made must be backed by further experimental data and, more important, this is a component which should be examined closely for possibilities of manipulation.

The only adequate simulation of rhizosphere biology that I know is the mechanistic model of Newman and Watson (1977) in which microbial abundance in the rhizosphere was simulated. The growth rate of micro-organisms at each point in soil was assumed to be controlled by the concentration of soluble organic substrate. The concentration of substrate changed due to 1) its production by the root and diffusion through soil, 2) its production in the soil by breakdown of insoluble organic matter, and 3) its use by micro-organisms. Some of the results of the simulation and the effects of varying certain parameters are given in Table 3.

In the Newman-Watson models, diffusion of substrates through wet soil was rapid enough for extensive microbial

TABLE 3

Predicted microbial concentrations at three distances from the root after 10 days by the simulation of Newman and Watson (1977)

Varied input parameter[1]	Microbial concentration ($\mu m\ cm^{-3}$)			Extent of rhizosphere[2] (mm)
	Distance from root			
	0	0.3 mm	1.8 mm	
Soil moisture †				
0.45 cm^3water cm^{-3}soil	234	23.5	4.3	> 4
*0.30	1509	14.5	2.2	0.7
0.20	7355	4.4	2.0	0.3
Exudation rate				
0.4 $\mu g\ cm^{-2}$day	12.5	4.5	2.2	0.4
*4.0	1509	14.5	2.2	0.7
Initial microbial concentration				
0.2 $\mu g\ cm^{-3}$	-280	12.5	1.1	> 4
*2.0	1509	14.5	2.2	0.7
20.0	1852	31.6	19.9	0.2

[1] Indicated parameters were varied and all others kept at standard.

[2] Distance at which microbial concentration was twice the initial value.

* "Standard" condition.

† -0.1, -0.3 and -1.0 bars matric potential, respectively.

development as far out as 4 mm. In drier soil (-0.3 bar) some exudate initially diffused into soil thus stimulating microbial growth, but as populations developed at the root surface they captured more of the exudate and much less diffused into soil. Maximum substrate concentrations 0.8-1.8 mm from the root were at approximately 8 h. At -0.3 bar soil matric potential, microbial abundance at the root surface was 15 times that at 0.1 mm and 430 times that at 0.8 mm at 10 days. With lower diffusion coefficients of substrates in soil, i.e. at -1 bar matric potential, still a moisture conducive to good root growth, the gradients of microbial abundance with distance from the root were even more steep. If this model can be validated, clearly it has practical use in predicting the distance from the roots at which propagules may receive enough substrate to germinate under various conditions. The limited diffusion of soluble substrate predicted by the model suggests that volatiles may be very important in stimulating microbial activity more than 1 to 2 mm from the roots.

In Newman and Watson's model a 10-fold difference in exudation led to a 120-fold difference in microbial abundance. Exudation therefore may be an area capable of manipulation. Newman and Watson stressed that much more study is needed of the time course of exudation from various parts of roots as they mature and age. The initial microbial population only slightly affects the final population at the root surface after 10 days. The greater multiplication occurring with the smaller initial populations suggests, however, greater scope for particular organisms to dominate and affect the species composition of the rhizosphere.

This model, like any model, needs experimental testing and possibly later refinement in critical areas. The data of Bowen and Rovira (1973) are remarkably consistent with it. However, its important predictions are themselves no proof, they indicate urgent areas for critical testing and further research. Simulations of this type have much to recommend them.

Root distribution and rhizosphere biology

Baylis (1975) indicated two extremes of root form - the 'graminoid' form (grasses and sedges) having very fine roots, often less than 0.1 mm in diameter and 'magnolioid' roots with their finest roots rarely less than 0.5 mm diameter. The concentration of roots in soil (L_v cm cm^{-3} soil) in grasses is up to 10 times that of species with roots approaching the magnolioid type, e.g. *Pinus* spp., avocado and citrus.

Fungal propagules with large reserves can grow several centimetres to a root, but small propagules may have to be adjacent to a root to colonize it. The distances that propagules can grow to roots and the concentration of roots produced by the plant will have a large bearing on the pathogen levels considered to be hazardous. By regarding the differing 'mobility' of pathogens as analogous to differing mobility of ions to roots, models developed for ion uptake by roots in soil may be adaptable to root colonization by propagules in soil. For example, from an analogy with ion uptake (Barley, 1970) one might expect relationships between numbers of colonization points and root density like thise of Figure 5. Large numbers of small propagules spread through soil or fewer larger propagules able to grow large distances to the root (and possibly of greater longevity in soil) are two efficient strategies employed by pathogens. Production of large propagules by pathogens of grasses and other densely rooting plants may be superfluous but large propagules may be very important for pathogens of trees in which inter-root distances are much greater.

Because colonized organic matter from roots (and top residues are sources of microbial inoculum for roots) rooting intensity should affect the rate of change of the microflora and build-up of suppressiveness on changing the cropping system. More rapid microbial changes would be expected under herbage and grass systems, compared with tree systems in the first few years, e.g. the root biomass in non-irrigated grass-pasture systems in the first year commonly ranges from 1.4-5.5 t ha^{-1} (Bray, 1963), but that of a high productivity plantation of *P. radiata* one year after planting in the field is only 0.24 t ha^{-1} (P.G. Zed and R. Boardman, unpublished results). Furthermore, the much finer roots of grasses and their much higher root concentration would lead to a more homogeneous distribution of the "new" microflora through soil than would young tree roots. If one is experimenting with plants to stimulate antagonists to pathogens of a succeeding crop, one should first consider plants with high rooting densities: e.g. grass roots stimulate high populations of *Phialophora radicicola* var. *graminicola* which is antagonistic to *Gaeumannomyces graminis* var. *tritici* (G.g.t.) (Slope *et al.*, 1978). It would be interesting to examine rapidity of build-up of suppressiveness to G.g.t. in continuous wheat soils as a function of growth conditions and root biomass in previous years.

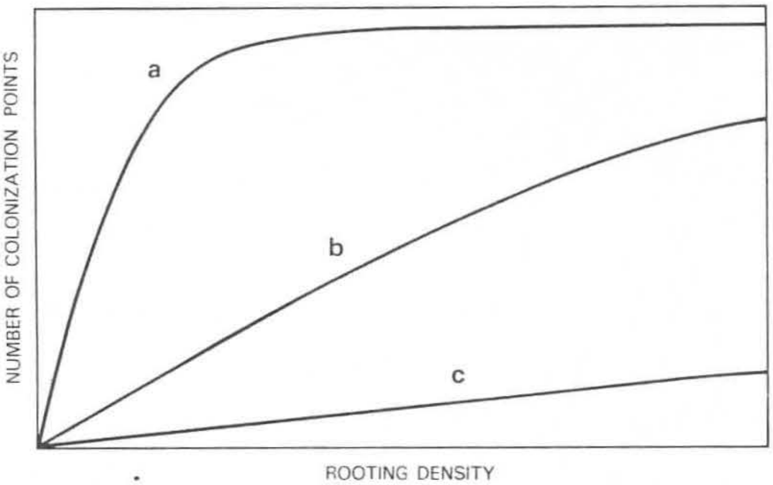

Fig. 5 Hypothesized colonization points with different rooting densities and low (constant) inoculum density of propagules growing over a) long distances to the root, b) moderate distances and c) very small distances (Bowen and Rovira, 1976).

Conclusion

In trying to control the growth of pathogens in soil and in the rhizosphere one must aim for conditions which will discourage the growth of the pathogen but encourage growth of antagonists and competitors. Simple methods are available for examining specific questions on the growth of organisms in soil and around roots and more of this type of approach would be valuable in defining ways in which we can manage particular organisms. In building up suppressiveness, antagonism, and active competition toward the pathogen, more emphasis should be placed on colonization of roots of previous crops. This involves not only examining conditions encouraging the desired organisms, but also examining plant aspects, such as root biomass and distribution.

The study of growth of organisms to the root and their growth in the rhizosphere should be integrated more directly with whole plant physiology (and vice versa), for manipulation of the plant may well affect the energy sources available for micro-organisms. As well as examining agronomic practices affecting particular organisms, more attention could be directed toward capitalizing on unique products from the roots to depress growth of pathogens and to enhance growth of inoculated antagonists "designed" to also use these products as energy sources.

There is a large reservoir of concepts and techniques in population biology, ecology and ecophysiology able to be transposed to rhizosphere biology. Some have been "tapped" already but these are relatively few and we could well avail ourselves of many more. I personally applaud the move toward simulation of growth of organisms in the rhizosphere because of the insight models can give to the way a system works, because of their indication of the most important parameters controlling the system, and because they can allow one to focus on the more important experiments to perform. The predictions must always be tested but the testing has more purpose and direction in it than experiments planned from personal "hunches". Such models have great potential in developing concepts of epidemiology and growth on roots and for this reason deserve more attention by microbiologists and plant pathologists.

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